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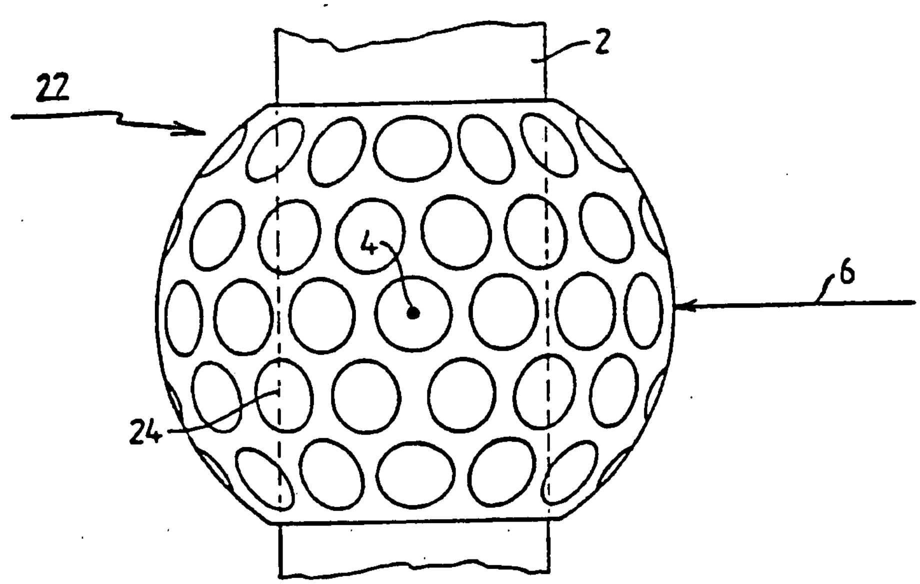

(57) Abstract

A flow cytometer and a method for determining properties of single cells or other particles (40) including passing stream of particles through a zone of analysis where a light source directs a beam of light (6) to perpendicularly intersect the stream of particles so that only a single cell (4) is exposed to the light beam (6). An array of optical fibers adjacent the zone of analysis collects the light refracted by the cells as each cell (4) passes through the zone of analysis. Each fibre is connected to a photomultiplier for converting the light to electrical signals which are analyzed by an electronic analysis unit to determine the particle properties. The angle at which the light is collected by said optical fibre is adjustable to permit more light to be collected to yield more information about the particle.

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FLOW CYTOMETERS

BACKGROUND OF THE INVENTION

The present invention relates to flow cytometers.

Flow cytometers (FCM) are instruments by which properties of single cells or other particles in suspension can be determined. Conventionally, an FCM consists of the following basic components:

i. A liquid flow system by which cells in suspension, which may be loaded with fluorescent dye, are transported in a vertical particle stream and passed singly, one after another, across a zone of analysis where they are exposed to an intense light beam. This zone may be located in open air or in a glass flow chamber;

ii. A light source and focussing system which directs a light beam (for example a laser beam) sharply focussed into the zone of analysis within the particle stream such that only a single cell will be exposed to the beam;

iii. An optical detection system, by which the scattered or fluorescent light pulses emitted by each cell at the moment when the cell passes across the beam, is collected, selected according to wavelength and converted into electronic pulses;

iv. An electronic analysis unit by which these pulses are processed and analyzed for the desired information about the cell characteristics which can be obtained from the light pulses.

A conventional optical detection system is shown schematically in Figure 1, which is a horizontal section through a flow chamber of an FCM.

In Figure 1, the flow chamber through which the particle stream passes is shown at 2, the section being taken at the point at which the incident light beam intersects the stream. The cell instantaneously exposed to the beam is shown at 4 and the incident light beam is shown at 6. The light pulses which are emitted from the cell 4 are collected perpendicularly to the incident beam 6 within a solid angle ( ) by a lens 8, then passed through a first beam splitter 10a. The light deflected by the first beam splitter 10a is passed through a color filter 12 onto a first photomultiplier PM_1 for transformation into electronic signals. The light transmitted through the first beam splitter 10a meets a second beam splitter 10b. The light respectively deflected and transmitted by the second beam splitter passes through further color filters 14, 16 to further photomultipliers, PM_2 and PM_3. Thus the light pulses are analyzed in three different parts of the wavelength spectrum.

This conventional detection system is disadvantageous in that each part of this system needs to be adjusted for correct location in three dimensions, and even with very experienced operators, initial adjustments and readjustments during measurement may involve several hours work. With systems effecting more than three color analysis, the use of a highly skilled operator is required for operation.

Further, with this conventional system, all analysis is restricted to the two dimensional plane in which the optical system is mounted. An analysis which could be carried on without such restriction would yield more information concerning the light scatter characteristics of cells, and a higher proportion of the omnidirectional, but normally weak, fluorescent light could be collected.

SUMMARY OF THE INVENTION

According to the present invention, there is provided an optical detection system in a flow cytometer, comprising an array of optical fibres which are located directly adjacent to the zone where the light from the cell is emitted, whereby the fibres act to collect emitted light.

At most, the ends of the fibres will be within a few millimeters from the cell.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be further described, by way of example only, with reference to the accompanying drawings, in which:

Figure 1 is a schematic of a conventional prior art optical detection system;

Figure 2 is a schematic horizontal cross--section through a flow chamber of a flow cytometer to illustrate the basic principles of the present invention;

Figure 3 is a similar horizontal section of a first practical embodiment of the invention;

Figure 4 is a side view of the embodiment of Figure 3;

Figure 5 is a horizontal section of a second practical embodiment of the invention; and

Figure 6 is a side view of the embodiment of Figure 5.

DESCRIPTION OF THE INVENTION

In accordance with the invention it has been determined that optical fibres can be used to collect directly the fluorescent or scattered light from the cell. A very simple mounting system for the fibres can be used which does not require a high accuracy in setting up. More particularly, the optical fibres may be held by the hand or fixed with a putty-like substance about 1 mm from the flow chamber and with this form of mounting the readings of scatter and fluorescence signals obtained have been found to have the same order of accuracy as achieved by a conventional optical system when set up in its optimum manner.

Figure 2 shows, schematically, a horizontal section through a transparent vertical flow chamber 2 through which the particle stream passes centrally, the section being taken at the point at which the light beam intersects the stream. The excited cell is shown at 4, and the incident light beam is shown at 6. An optical fibre which directly collects the emitted light is shown at 20. In the configuration shown in Figure 2, the optical fibre 20 collects light emitted from the cell 4 within a solid angle α along an axis inclined at an angle β to the incident beam 6. As will be apparent, simply by moving the fibre 20 toward or away from the chamber 2, the measured solid angle α can be changed; a

similar effect can be obtained by altering the size of the light-acceptance aperture by means of an aperture mask at the end of the fibre. The fibre can also be moved in order to change the angle β relative to the incident beam 6. The choice of angles α and β is not given in conventional detection systems where both angles are fixed.

In one practical embodiment as shown in Figures 3 and 4, a part-spherical shell 22 is mounted around part of the flow chamber 2, the center of the sphere being coincident with the instantaneously excited cell 4 in the chamber 2. Thus, the center of the shell 22 is coincident with the point of intersection of the incident light beam 6 with the particle stream. The beam 6 passes through an appropriate opening 23 in the shell 22. Holes 24 are formed through the wall of the shell 22, the axis of each hole 24 lying on a different radial axis of the shell 22 so that each hole 24 faces toward the excited cell 4. A group of optical fibres is provided (not shown), the fibres leading to one or more photomultipliers. The ends of the fibres can be removably plugged into any one of the holes 24 in the shell 22 to enable readings to be taken at selected points around the cell 4, in other words at different angles of β with the possible variation of this angle not only being in the plane of Figure 2 but also in planes inclined to that of Figure 2. A compromise has to be made between the desire for high angular resolution by small solid angles and the need to collect sufficient amounts of light. Therefore, in practice, the solid angle α of light collection for each photo-

multiplier also needs to be variable. Possible methods of varying the solid angle include the following:

a. Different sizes of holes 24 for fitting different diameter fibres. This would require a predetermination of angles of interest for the scatter light analysis, where the angle of resolution is important, the remaining angles being free for larger size fibres collecting the omnidirectional fluorescent light.

b. Fibre fittings for allowing variation of depth of fibre plugging, thus varying the angle of light acceptance by the distance of the light acceptance aperture from cell 4.

c. Photomultipliers for allowing collective entry of many fibres, so that for weak fluorescent light, the light from different directions may be collected by several fibres and directed into one photomultiplier.

Since fibres are relatively inexpensive, the fibres may be fixedly mounted in the shell 22. In this embodiment each hole 24 is non-removably plugged with a fibre, with the selection of light analysis angles being obtained by plugging the other ends of the relevant fibres into selected photomultipliers. This would facilitate the precision-setting of all fibres on the shell 22 and thus reduce alignment problems.

The shell 22 may be supported by a mounting system which allows adjustment of the position of the shell 22 in all directions relative to the flow chamber.

Alternatively, the shell 22 may be mounted by a precision lock in a fixed position relative to the flow chamber, to thereby avoid the necessity of having to align the system subsequent to manufacture.

It is to be noted that the flow chamber 2 is not of conventional rectangular cross-section, but in the embodiment shown is of circular cross-section, the chamber being of cylindrical form. Alternatively, the chamber may be of spherical form, with the entry and exit areas of the incident light beam being flattened. In this case all non-perpendicular transitions of light through the interface between glass and air would be avoided. However, the use of a flow chamber is not essential, and the system shown in Figures 3 and 4 can be used in an FCM in which the particle stream moves through open air.

In another practical embodiment, as shown in Figures 5 and 6, the chamber 2 extends through a parabolic reflective shell 30 with the instantaneously excited cell 4 being at the focus of the parabola. This parabolic shell 30 is closed by a circular plate 32 the center of which is apertured for passage of the incident light beam 6 onto the cell 4 at the focus of the shell 30. The shell itself is provided with an aperture 33 in alignment with the central aperture in the plate to permit exit of the light beam 6. Holes 34 are formed through the plate 32 in a number of concentric rows. With each hole 34 being directed perpendicularly to the plane of the plate 32, i.e. parallel to the light beam 6.

Due to the parabolic form of the reflective shell 30, the scattered and fluorescent light will be reflected parallel to the axis of the parabola, that is at right angles to the plate 32 and parallel to the incident beam 6. Optical fibres leading to photo-multipliers can be plugged into selected ones of the holes 34. As will be apparent each concentric row of holes will be associated with light scattered at the periphery of discrete cone angles, and fibres plugged into the respective rings will collect light at different points around the relevant cone angle. In effect, the use of the parabolic shell enables the collection of the light emitted all around a certain cone angle and this can be detected with high angular resolution despite an overall large area of light collection due to rings of fibres.

Instead of using a reflective paraboloid separate from the flow chamber, it might be advantageous to shape the whole flow chamber accordingly and provide the chamber with a reflective coating. This would avoid non-perpendicular transition of light through interfaces of media with different refractive indices (glass-air) and thus avoid reflection and beam-shift problems.

The use of a fixed or movable mounting system for the shell itself and of removable or non-removable fibres as discussed in connection with the previous embodiment, applies to this embodiment also.

In the two practical embodiments described, color discrimination filters can be associated with the fibres, the filters preferably being positioned at the point where the fibres enter the housing of the photo-multipliers.

If light polarization studies are to be performed, polarizing filters must be applied before the light enters the fibres because of the depolarizing effect of fibre light conductors.

The use of optical fibres to directly collect the emitted light provides enhanced flexibility of measurement in relation to that of a conventional optical system, and permits easier setting up of experiments. More specifically, the main advantages of the described systems are:

i. Reduction of optical alignment problems;

ii. Reduced need for highly skilled personnel for operating the system;

iii. Reduced cost of flow cytometers;

iv. Increased versatility for sophisticated non-routine investigations on cell discrimination.

While illustrative embodiments of the subject invention have been described and illustrated, it is obvious that various changes and modifications can be made therein without departing from the spirit of the present invention which should be limited only by the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A flow cytometer comprising:

means for transporting a stream of particles having cells in suspension such that each cell passes singly through an analysis zone;

a light source for directing a light beam that perpendicularly intersects said particle stream at said analysis zone such that only a single cell will be exposed to said light beam;

an optical detection means including at least one optical fibre located adjacent said analysis zone for collecting the light emitted by each cell as each cell passes through said analysis zone; and

electronic means connected to said optical fibres for converting the light collected by said optical fibres into electrical impulses and analyzing said impulses for the desired information.

2. The flow cytometer of Claim 1, wherein said optical detection means includes an array of optical fibres.

3. The flow cytometer of Claim 1 or 2, wherein each of said optical fibres collects the light emitted by the cells within a collection angle defined by the cell and the outer perimeter of the optical fibre and at an incident angle defined by the light beam and the longitudinal axis of the optical fibre.

4. The flow cytometer of Claim 3, wherein said collection angle is adjustable by changing the distance between said cell and said optical fibres.

5. The flow cytometer of Claim 3, wherein each of said optical fibres include an aperture mask for adjusting the collection angle.

6. The flow cytometer of Claim 1, 2 or 3, wherein said optical detection means includes a shell mounted around the analysis zone, said shell having an opening for allowing said light beam to pass through said shell into said analysis zone, said shell further including a plurality of apertures for receiving at least one optical fibre.

7. The flow cytometer of Claim 6, wherein said shell is substantially spherical in configuration with the center of said sphere being coincident with the cell in the analysis zone and wherein the axis of each aperture lying on a different radial axis of said shell so that each aperture faces toward the cell in the analysis zone.

8. The flow cytometer of Claim 6, wherein said shell is partially parabolic in configuration including a partial parabolic reflective surface ending at a flat circular plate positioned perpendicular to said beam of light wherein the focus of said parabola being coincident with the cell in the analysis zone, said flat circular plate having said plurality of apertures and said opening for the light beam.

9. The flow cytometer of Claim 8 wherein said apertures are formed in concentric rows with said opening in the center thereof, each of said apertures being directed perpendicular to the plane of said circular plate.

10. The flow cytometer of Claims 6, 7, 8, or 9, wherein said optical fibres are removably mounted within said apertures thereby permitting light to be collected at different incident angles.

11. The flow cytometer of Claims 6, 7, 8, or 9, wherein said apertures are of several different sizes for receiving optical fibres of different diameters thereby varying the collection angle.

12. The flow cytometer of Claims 6, 7, 8, or 9, wherein said optical fibres are adjustably mounted within said apertures allowing variation in the depth said optical fibres are received in each aperture thereby varying the collection angle by varying the distance between said cell and said optical fibre.

13. The flow cytometer of Claims 6, 7, 8, or 9, wherein a plurality of said optical fibres are connected to a photomultiplier for allowing light from different incident and collection angles to be analyzed together.

14. The flow cytometer of Claims 6, 7, 8, or 9, wherein a plurality optical fibres are rigidly secured in the apertures of said shell and a variation in said incident and collection angles being obtained by connecting the optical fibres to a plurality of selected photomultipliers.

15. The flow cytometer of Claims 6, 7, 8, or 9, wherein said shell is adjustably mounted to allow adjustment of the position of said shell relative the analysis zone.

16. The flow cytometer of Claims 6, 7, 8, or 9, wherein said shell is secured in a fixed position relative to said analysis zone.

17. The flow cytometer of Claim 1, wherein said transporting means includes a flow chamber through which said particle stream passes, said analysis zone being within said flow chamber.

18. The flow cytometer of Claims 6, 7, or 8, wherein said transporting means includes a flow chamber through which said particle stream passes, said analysis zone being within said flow chamber, said flow chamber being within said shell.

19. The flow cytometer of Claim 1, wherein said transporting means includes a flow chamber through which said particle stream passes, said analysis zone being within said flow chamber, said flow chamber having a partially parabolic surface having a reflective coating thereon, said partial parabolic surface ending at a flat circular plate having an opening for allowing said light beam to pass through.

20. The flow cytometer of Claim 19, wherein said flat circular plate of said flow chamber includes a plurality of apertures for receiving said optical fibre.

21. The flow cytometer of claims 6, 7 or 8 wherein said electronic means includes at least one photomultiplier.

22. The flow cytometer of Claim 21 further including a color discriminating filter connected to each of said fibres.

23. The flow cytometer of Claim 22 wherein said color discriminating filters are positioned at the point where said fibres enter said photomultipliers.

24. A method for determining the properties of particles comprising:

passing a stream of particles through a zone of analysis;

directing a light beam perpendicularly intersecting same stream of particles, said light being refracted by said particles as each particle passes through the zone of analysis;

collecting the refracted light with at least one optical fibre;

electronically analyzing said collected refracted light to determine the desired information.

25. The method of Claim 24, wherein said stream of particles includes cells in suspension.

26. The method of Claim 25, wherein said cells are singly passed through said zone of analysis and said light beam is directed such that only a single cell is exposed to said beam.

27. The method of Claim 24, further including the step of adjusting the distance between said optical fibre and said zone of analysis.

28. The method of Claim 24, wherein said refracted light is collected by an array of optical fibres.

29. The method of Claim 28, wherein said array of optical fibres are removably and adjustably mounted to a shell surrounding said zone of analysis.

30. The method of Claim 28, wherein said array of optical fibres are fixedly mounted to a shell surrounding said zone of analysis.

31. The method of Claim 24, wherein said zone of analysis is within a flow chamber.

32. The method of Claim 28 or 29, further including reflecting the light refracted by said particle off the inner surface of said shell and wherein said array of optical fibres collects said reflected light.

33. The method of Claim 24, further including the step of connecting said optical fibre to a photo-multiplier for connecting said collected light into a series of electrical pulses.

PRIOR ART

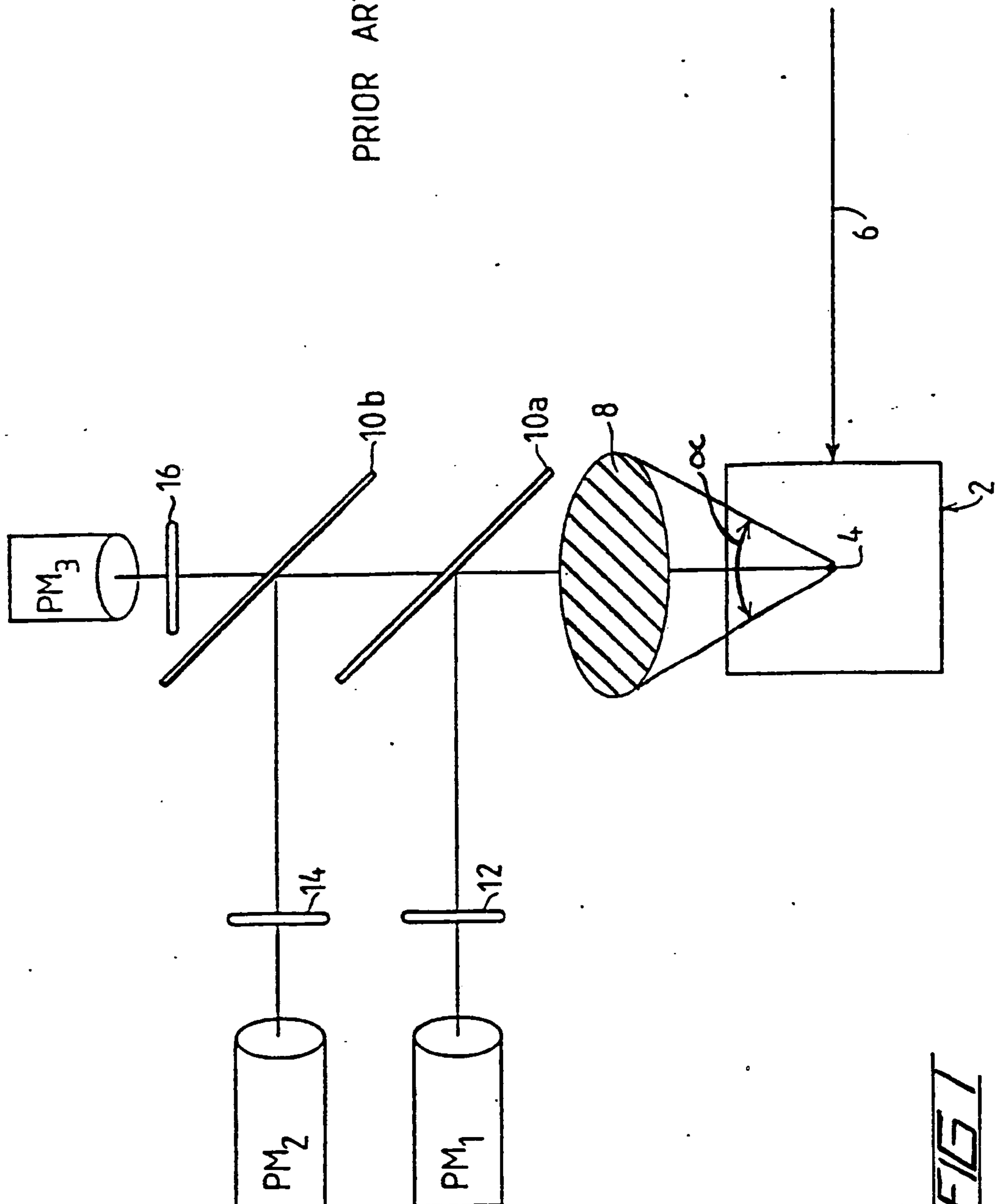


FIG 1

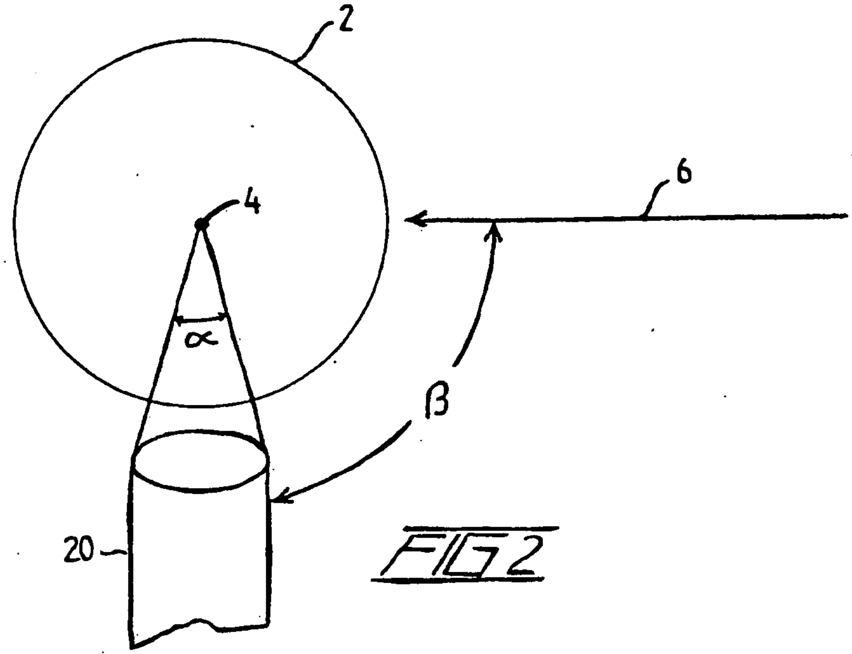


FIG 2

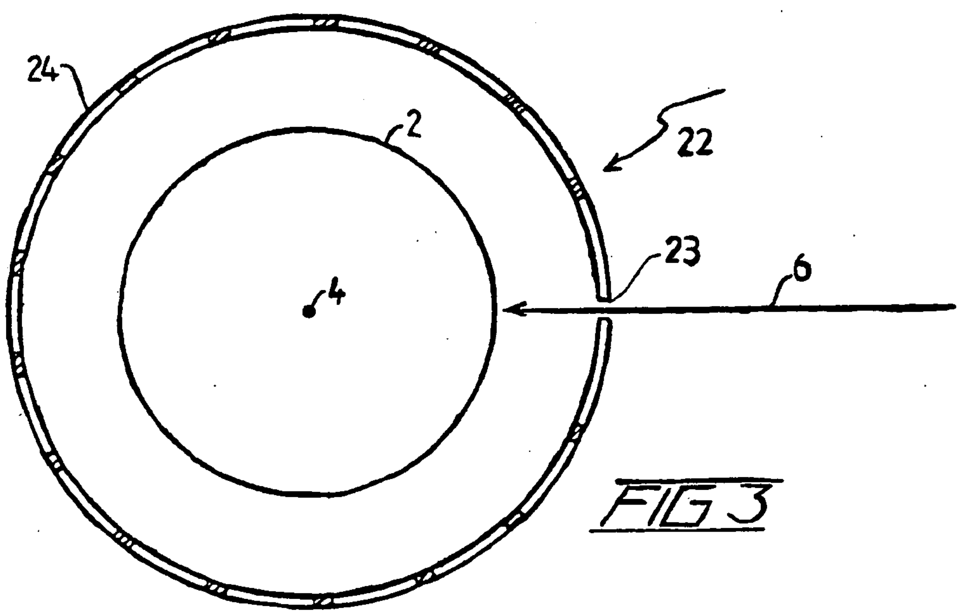


FIG 3

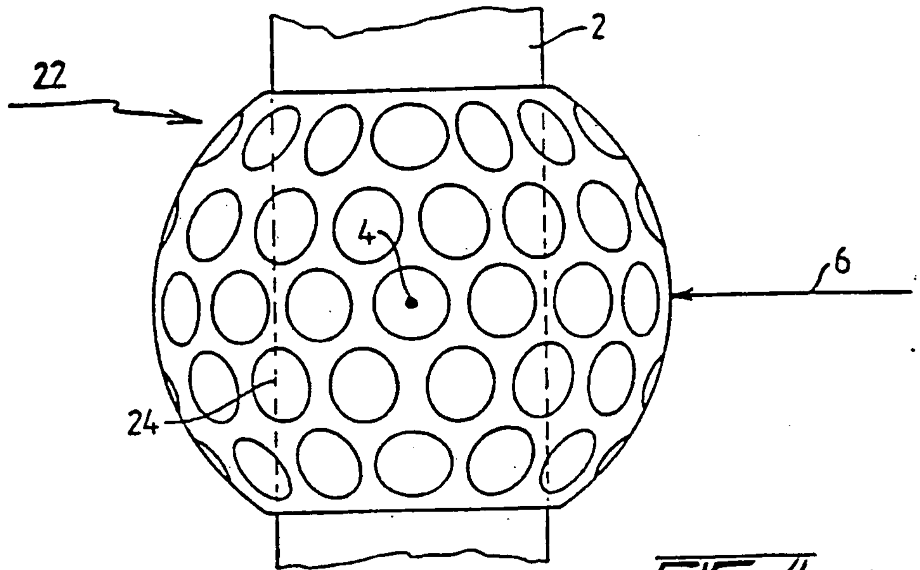


FIG 4

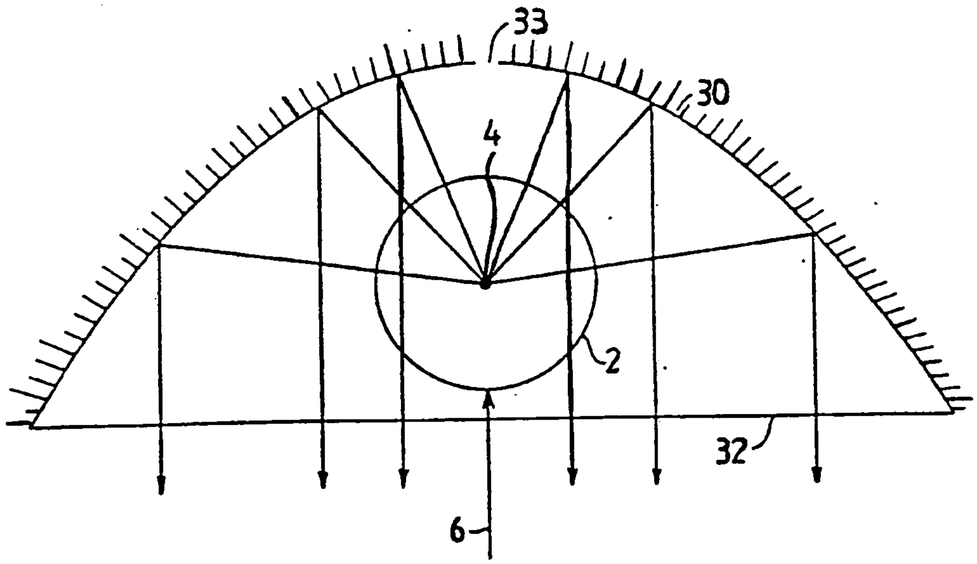
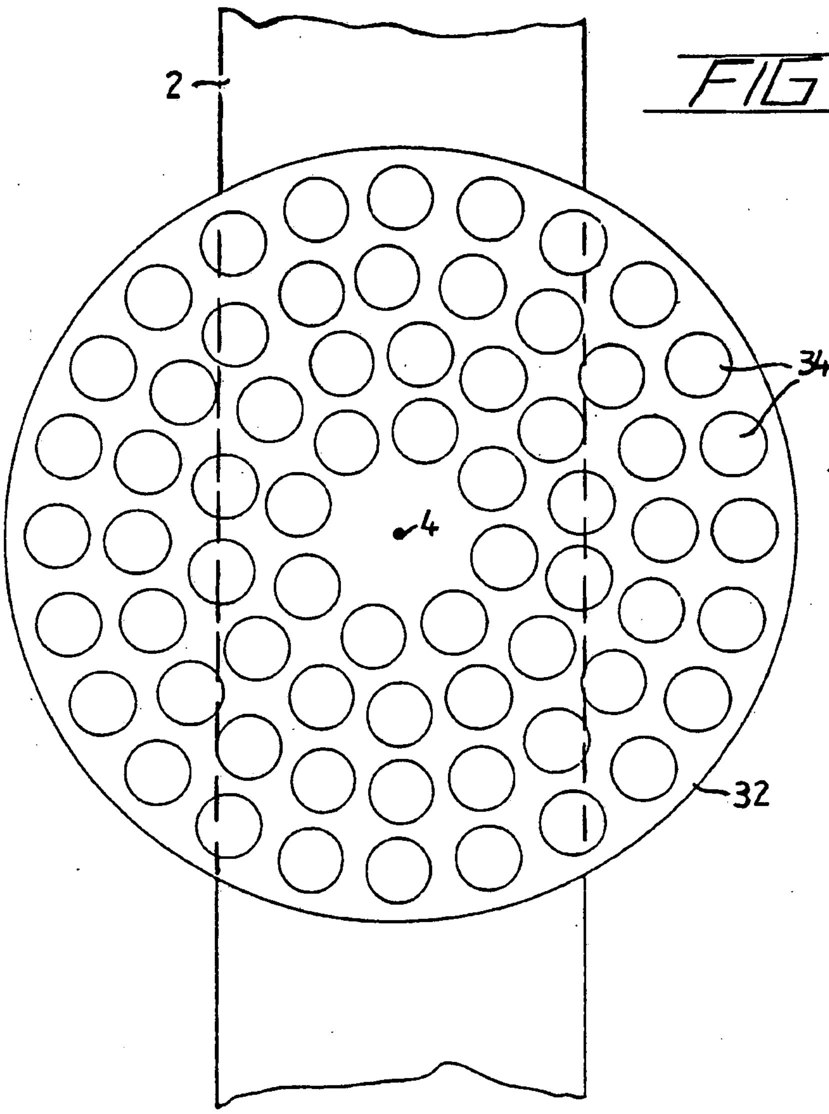


FIG 5

FIG 6



INTERNATIONAL SEARCH REPORT

International Application No PCT/US85/00314

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

INT. CL.[4] G01N 21/00
U.S. CL. 356/73, 340

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
US	356/39, 72,73,336,37,338,339,340,341,343 250/227

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
A	US,A, 3,781,112, Published 25 December 1973, Groner et al.	1-33
A	US,A, 4,101,276, Published 18 July 1978 Anderson	1-33
Y	US,A, 4,175,865, Published 27 November 1979 Horvath et al.	1-33
Y	US,A, 4,200,802, Published 29 April 1980 Salteman et al.	1-33
Y	US,A, 4,201,471, Published 6 May 1980 Pitt et al.	1-33
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IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
7 May 1985	13 MAY 1985
International Searching Authority [1]	Signature of Authorized Officer [20]
ISA/US	Richard A Rosenberger

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